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AD843482

TRANSLATION NO. 3152

DATE: 25 October 1960

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TRYPTOPHAN-TO-NICOTINIC-ACID METABOLISM IN CHILDREN WITH FEBRILE CONVULSIONS

Acta Vitaminologica
(Vitaminology Procedings)
Vol 20, Nº 4 pp 135-142, 1966

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Over the last several years, since Adams and his colleagues reported in 1954 (1) that children exhibiting a convulsive syndrome were found to have been fed with artificial milk having a low Vitamin B_6 content, and that these children's urine contained xanthurenic acid, several authors have investigated the modifications of tryptophan metabolism in children with convulsions of various kinds.

This research has shown that in a great many such cases there is increased elimination of xanthurenic acid following ingestion of tryptophan. Quantitative data on the other metabolytes of this amino acid are scant, however, and those available are not always in agreement. Some show an increase in levels of kynurenic acid or the kynurenines, while others indicate a rise in other metabolytes, as shown on Table I. This Table also provides an indication of the particular morbose conditions investigated by each author, and the tryptophan doses used in testing.

A look at the Table will show that the data from these various authors are not readily adaptable to comparison, both because of the variety of clinical situations explored and because of differences in methods of evaluation. Moreover, the literature is almost wholly lacking in quantitative data on urinary elimination of the kynurenines and their derivatives under normal conditions. We recently published some of our own findings on children during the first year of life (11).

The purpose of the research reported in this paper was to obtain quantitative figures on urinary elimination of metabolytes in the tryptophan-nicotinic-acid line in children with

febrile convulsions, both under base conditions and after a dose of the amino acid and after treatment with pyroxidine. Some additional observations were also made on cerebropathic children whose clinical symptomatology showed flexion spasms.

MATERIALS AND PROCEDURES

We examined 10 children with febrile convulsions diagnosed on the basis of clinical data and EEG, ranging from 8 to 36 months of age, and 4 cerebropathic children ranging in age from 75 days to 28 months.

All the children were patients in the Pediatric Clinic at the University of Milan.

In 24-hour urine samples, generally taken from the 48th to the 72nd hour after the onset of the febrile convulsions, we measured the amounts of the following tryptophan metabolytes: kynurenine, 3-OH-kynurenine, acetyl-kinurenine, kynurenic acid, and xanthurenic acid. We then administered an oral dose of l-tryptophan (5g per 1.73 sq. meters of body surface), and collected the urine for the next twenty-four hours, analyzing it for the same metabolytes.

The dosage procedures we used were the following:

1) The Brown-Price method (12) for kynurenine and acetyl-kynurenine;

2) the Musajo-Coppini method (13) for xanthurenic acid;

3) Brown's method (14) for 3-OH-kynurenine;

4) the Satoh-Price method (15) for kynurenic acid.

In order to make sure that the urine contained no substances that might mimic the metabolytes we were studying in colorimetric qualities, we preceded our quantitative analysis of the tryptophan metabolytes with monodimensional paper chromatographic separation and observation of the chromatogram after Wood's procedure (15 bis).

FINDINGS AND DISCUSSION

On Table II we have summarized the data on urinary elimination of the kynurenines (kynurenine, acetyl-kynurenine, and trioxykynurenine), both under base conditions and after tryptophane ingestion in our febrile-convulsive children.

There is quite clearly a marked diversity of behavior from one subject to the next from the quantitative aspect. In all subjects, at least one of the three kynurenines is eliminated in comparatively high quantity. Furthermore, these values as a

TABLE I - Urinary elimination (mg/24 hrs) of tryptophan metabolytes in children with convulsions of various kinds and in hypsarrhythmic children. Remarks on pyroxidine and ACTH treatment. Data from the literature.

Nº of cases	Bib. ref.	Age	Syndrome	Tryptophan (DL or L) administered (g/kg)
3	(1)	3-7 m	Convulsions	Not indicated.
4	(2)	13-42 m	Febrile convulsions	0.54 (DL)
9	(3)	3-9 m	Infantile spasms with hypsarrhythmia	0.54 (DL,L)
10	(3)	2-9 y.	Convulsive symptoms of obscure origin	0.54 (DL, L)
5	(4)		Infantile spasms and mental retardation beginning at 4-6 mos.	Not indicated.
18	(5)		Infantile spasms with oligophrenia: 6 symptomatic (a), 12 cryptogenetic (b)	0.4 (DL); 0.2 (DL)
7	(6)		Convulsions of various kinds, resistant to anti-convulsants	0.2 (L)
11	(6)		Hypsarrhythmia	
13	(7)	$3\frac{1}{2}$-26m	Infantile spasms of obscure origin, with symmetrical myoclonus or brief tonic contraction with hypsarrhythmia. Symptoms: duration, 3-18m; age of onset, 2 to 18 months.	0.1 (L)
20	(8)	11-30m	Febrile convulsions, EEG negative.	5g x 1.73 sq.m. body surface. (L)
6	(9)	$3\frac{1}{2}$ 21 m	Infantile spasm syndrome, hypsarrhythmia	5g x 1.73 sq.m. body surface (L)
10	(10)	5-36 m	Hypsarrhythmia	0.1 (L)

N.B. The Roman numerals used for abbreviation indicate:
I - Kynurenine II - 3-OH-kynurenine III - Kynurenic acid
IV - 3-OH-antranylic V - Acetyl kynurenine VI - 0-ami[illegible]ppuric

Tryptophan metabolytes		Remarks on treatment with pyroxidine (P) or ACTH (A)
Xanthurenic acid	Others	
Present		
1-10 mg		P- I case treated; absence of xanthurenic acid.
Increased		A - clinical and EEG improvement seem to coincide with xanthurenic acid normalization
In 6 normal subjects		P - clinical improvement.
	Normal	P - clinical improvement.
(a) normal levels (b) abnormal levels (over 5mg in 6 subjects		P- 3 cases showed no certain B_6 deficiency. A-in 2-3 wks. spasms stopped or reduced; EEG improved; xanthurenic acid level lowered.
In 1 subject 0; in 6 subjects 1-17.5mg (12-hour urine)		P - no clinical or EEG response, except in 1 case.
Mild xanthenuria (2.1-9.5mg). Absent in 3 cases (12-hour urine).		P - favorable in 1 case. Visible improvement in 2, with fewer tonic seizures.
Increased in several cases.	Normal I, II, III, IV.	P - cerebral symptoms not affected. A - no clinical effect in 3 out of 10 cases.
Increased by comparison with I.	I and derivatives up; normal in 14 cases after cessation of fever.	
Increased	I and III up. II,V often up.	P- metabolyte elimination unchanged. A-metabolyte elimination normalized, 4 cases.
Up (50-240 gemma/cc)	Normal I,II and VI.	

whole are higher than those we obtained from normal subjects, and higher than those reported by other writers, even making allowances for the varying conditions under which the amino acid is administered, the differences in age, etc (See Table III).

Elimination of kynurenic acid and xanthurenic acid, on the contrary, does not appear to deviate noticeably from the norm. It should be noted, however, that these compounds were measured in only a limited number of samples, and that therefore we can make no definitive assertion on this score.

TABLE II - Urinary elimination (mg/24 hrs) of tryptophan metabolytes (*) spontaneously and after feeding with the amino-acid in subjects with febrile convulsions.

Subjects' initials	Age (mo)	Wt. (Kg)	Kynurenine		Acetyl-K.		3-OH-K.	
			Base level	After ingestion	Base level	After ingestion	Base level	After ingestion
C.A.	19	11.4	5.22	9.86	0.23	2.60	4.82	77.01
A.C.	8	7.7	0.82	11.25	0.22	2.57	3.30	48.58
F.F.	18	10	59.53	34.83	0.94	1.88	6.83	6.18
S.C.(**)	21	12	7.87	9.86	0.90	0.79	0.50	3.84
S.D.	36	15	243.7	321.2	5.27	11.66	46.06	36.27
C.A.	19	9.6	—	38.25	1.35	9.15	—	3.23
D.O.A.	24	10	127.3	293.9	2.29	9.49	6.67	7.71
P.G.	14	9.2	37	100	2.28	6.17	6.27	15.52
V.R.	11	10	13.7	26.65	1.40	2.90	2.05	6.25
L.L.	24	12	17	64.8	0.7	6.17	2.35	9.06

(*) Figures underlined are higher than the maximum levels we encountered in normal subjects under 12 months of age.
(**) Convulsions occurring 12 hours prior to start of the experiment.

After treatment with pyroxidine, given in 50- to 100-mg doses intramuscularly for 4 to 6 days, beginning after the first two urine collections, we found a reduction in urinary elimination of kynurenine, both at base levels and after ingestion, to levels that may be considered normal.

These observations, which substantially confirm our earlier measurements with the chromatographic method, may be explained on the basis of the possible existence in febrile-convulsive children of a condition of latent apyridoxinosis due to accelerated consumption of Vitamin B_6 in the tissues, which helps to bring on the convulsive symptoms, and which is probably

TABLE III - Urinary elimination (in mg/24 hrs) of tryptophan metabolytes, spontaneously and after ingestion of the amino acid, in healthy children under 4 yrs. old.

Authors & Bibl.ref.	No. cases and ages	Tryptophan dosage	Kynurenine	3-OH-Kynuren.	Acetyl Kynuren.	Kynurenic acid	Xanthurenic acid
Vasella et al. (19)	22 under 20 mos.	100mg/kg L	(12.4)	(1.8)		(3.2)	(0.9)
Dahler (20)	12 7 days to 8 mos.	500 mg/kg DL	10.7-74.4				0 - 18.4
Careddu et al. (11)	17 1 to 12 mo	--	0.19-27.7 (2.32)	0-1.05 (0.75)	0.02-0.84 (.17)	0 - 4.75 (1.81)°°	1.5 (1 case)
	15 1 to 12 mo	$5g/1.73m^2$° L	0.38-63.0 (8.41)	0-2.49 91.01)	0.09-1.80 (0.50)	0-14.37 (6.77)°°	3.1-3.8 (2 cases)
Kawamura (21)	4 3 to 4 yrs	--	2.75-7.42			2.07-3.02	0.31-1.84
		2g L	9.34-24.99			5.85-13.66	2.49-7.31

N.B. - On this table we do not show the data on measurements of xanthurenic acid levels alone; for these figures, we refer the reader to one of our earlier publications (11). Excretion levels shown in parentheses indicate averages. The indication (°) means square meters of body surface; (°°) means analyses performed on seven subjects.

TABLE III.

precipitated by the febrile manifestation.

This hypothesis could be tested by means of experiments similar to those described here performed on a group of children with fever, but no convulsions. In these cases, it will be recalled, one of us had already shown by paper chromatography that kynurenine elimination is slightly higher than in normal children, but never touches the levels of those with febrile convulsions (8).

In adults, Dalgliesh (16) reports that the febrile state involves a pyroxidine deficiency condition, masked by increased kynurenine elimination after elimination of tryptophan. His findings, however, are not confirmed by the observations of other authors (17).

A Vitamin B_6 deficiency could bring about increased elimination of xanthurenic acid in the urine after oral ingestion of tryptophan. In our subjects, we noted an almost universal increase in elimination of kynurenine, whereas in the few cases in which we studied it, xanthurenic acid elimination was normal.

We encountered more difficulty in interpreting the findings on the group of four children (Table IV), three of whom were cerebropathic with EEGs indicating the existence of organic brain damage. In the single case of hypsarrhythmia, in which the pathogenesis was probably dysmetabolic, we found alterations in the tryptophan metabolism of the same type as those described above by one of us as well as others (Table I), and demonstrated their reversibility under ACTH therapy.

In conclusion, we believe it fair to assume, on the basis of the results of our quantitative measurements of kynurenines in children with febrile convulsions, the existence of a latent Vitamin B_6 deficiency, which is revealed upon ingestion of tryptophan. These data, which agree with those obtained earlier by one of our number using semi-quantitative procedures (paper chromatography), might be further corroborated by other factors indicating a Vitamin B_6 deficiency, such as the drop in the transaminase count (and particularly in the aspartic fractions) in the erythrocytes, which has recently been found to be a sensitive index to Vitamin B_6 deficiency (18).

It is, however, not so easy to explain whether there is a relationship between the very serious nervous alterations which always underlie flexion spasm malady and the changes we observed in the tryptophan metabolism in the presence of this cerebropathic condition.

We have found, in fact, that the metabolic disturbances are not affected by pyroxidine, as they are by treatment with

TABLE IV. - Urinary elimination (in mg/24 hrs) of tryptophan metabolytes, spontaneously and after ingestion of the amino-acid, in cerebropathic subjects.

Subjects' no. and initials	Age mos	Weight (kgs)	Kynurenine		Acetyl-Kynuren.		3-OH-Kynuren.		Kyn. Acid		XanthurenicA.	
			Base levels	After ingestion	Base levels	After ingestion	Base levels	After ingestion	Base level	After ingestion	Base levels	After ingestion
1. L.I.	28	10.600	1.70º	53.92	0.43º	7.45	0.51º	16.01	2.12º	10.80	0	0
2. M.C.	21	5.280	0.42	34.85	0.06	11.06	0.02	2.95	-	-	-	-
3. B.G.	11	8.500	0.38	0.30	0.22	0.07	1.03	0	1.05	3.50	0	1.88
			0.09	1.28	0.04	1.18	0	0	1.40	11.28	0	4.94
4. R.M.	9	9.000	0.79 º	1.61	0.21 º	0.47	0.98 º	0	1.16º	21.74	0	7.59
			0.41	0.89	0.34	0.64	0.38	0.31	1.80	7.42	0	5.27
			0.82	1.60	0.14	0.11	0.13	0.11	1.46	2.50	0	1.63

º - Average of the two base levels taken at 24-hour intervals.

N.B. Diagnosis for each subject was as follows: Nº1 - cerebropathy with grave epilepsy; Nº2 - Spasms in flexion; Nº3 - Grave cerebropathy with microcephaly and atrophy of the optic nerves; Nº5 - Hypsarrhythmia.
For subjects 3 and 4, the second line refers to a test made 19 days after the first, after administration of 10 units ACTH per day for 15 days. For subject 4, the third line refers to a test made 40 days after the first, after administration of 10 units of ACTH per day for 36 days.

ACTH and cortisone.

In this case, the problem is tied in with that of the influence of corticotropine and corticoid treatment in certain cerebropathic conditions of the degenerative or sub-acute degenerative type. One might, perhaps, project the hypothesis that in these cases the effect of the corticosteroids and the ACTH is produced by means of activating enzyme systems, either in the nerve tissue or in other tissues, with an improvement in the clinical condition of the patient and the normalization of tryptophan metabolism.

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SUMMARY

NICOTINIC ACID TRYPTOPHAN METABOLISM IN CHILDREN WITH FEBRILE CONVULSIONS

In a group of 10 subjects with febrile convulsions, urinary elimination of Kynurenines (kynurenine, acetyl-kynurenine, and trioxykynurenine), both spontaneous and after ingestion of l-tryptophan (5g per 1.73 sq. meters of body surface), was found to be greater than that measured in normal subjects, even allowing for pronounced behavioral variations among subjects.

In a single hypsarrhythmic subject, tryptophan metabolism alterations regressed in the course of ACTH therapy.

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BIBLIOGRAPHY

(1) ADAMS, J.D. et al. - Am. J. Dis. Child., 1954, 88,623.
(2) RABE, E.F. and PLONKO, M. - Am. J. Dis. Child., 1956, 92, 382.
(3) JEUNE,M. et al. - Pédiatrie, 1959, 14, 853.
(4) COCHRANE, W.A. - 19th Internat. Cong. Ped., Montréal, 1959 (Scientific Program, p 11).
(5) BOWER, B.D. - Proceedings of the Royal Society of Medicine, 1961, 54, 540.
(6) SEGNI,G. and GANDULLA, E. - Min. Ped., 1962, 14, 1095.
(7) BO HELLSTROM, VASSELLA, F. - Acta Pediatrica, 1962, 51,665.
(8) CAREDDU, P. et al. - Pédiatrie, 1962, 17, 359.
(9) CAREDDU, P. et al. - La Pediatria, 1962, 3, 363.
(10) FOIS, A. and LECCHINI, L. - Riv. Clin. Ped., 1963, 72, 182.
(11) CAREDDU, P. et al. - Acta Vitamin,. 1964, 18, 241.
(12) BROWN, R.R. and PRICE, J.M. - J. Biol. Chem., 1956,219,985.
(13) MUSAJO, L. and COPPINI,D. - Experientia (Basel)1951,7,20.
(14) BROWN, R.R. - J. Biol, Chem., 1957, 227, 649.
(15) SATOH, K. and PRICE, J.M. - J. Biol.Chem.,1958, 230, 781.

(15 bis) GINHOULIAC, E. and TENCONI, L.T. - Acta Vitamin., 1951, 5, 246.
(16) DALGEISH, C.E. and TEKMAN, S. - Bioch. J., 1954, 56, 458.
(17) MUSAJO, L., BENASSI, C.A. and PARPAJOLA, A. - Clin. Chir. Acta, 1956, 1, 229.
(18) CHENEY, M., SEBRY, Z.I. and BEATON, G.H. - Am.J.Clin.Nutr., 1956, 16, 337.
(19) VASSELLA, F., HELLSTROM, B., and WENGLE, B. - Pediatrics, 1962, 30, 585.
(20) DAHLER, R.P. - Ann. paed., 1963, 200, 347.
(21) KAWAMURA, M. - Vitamins, 1965, 32, 111.